

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claims 1-77 (canceled).

Claim 78 (currently amended): A method for preparing a protein having a correctly folded human insulin precursor, said method comprising:

expressing a recombinant protein comprising, from N-terminus to C-terminus,

a first peptidyl fragment which has of about 20 amino acids in length to 92 amino acids in length having an amino acid sequence which is at least 60% identical to [[a]]the sequence of SEQ ID NO:1 of the same length as the first fragment, at least through residues 1 - 20 of SEQ ID NO:1 and wherein the first fragment is capable of being bound by an anti-hGH antibody;

a second human insulin precursor peptidyl fragment which is a human insulin precursor comprising the human insulin A chain and the human insulin B chain[[],]; and

an arginine or lysine residue or at least one cleavable peptidyl fragment linking the first peptidyl fragment and second peptidyl fragments the human insulin precursor fragment[[],];

wherein the first peptidyl fragment is capable of increasing the yield of the bioactive conformation of the insulin precursor formed upon contact of from contacting the recombinant protein with a chaotropic auxiliary agent as compared to the yield of the bioactive conformation of the insulin precursor formed from contacting the same recombinant protein lacking the first peptidyl fragment with the chaotropic agent; and

contacting the recombinant protein with an aqueous medium comprising the chaotropic agent[[; and]],

whereby the protein is correctly folded.

Claim 79 (currently amended): [[A]]The method according to claim 78, wherein the aqueous medium comprises at least one chaotropic auxiliary agent.

Claim 80 (currently amended): [[A]]The method according to claim 78, wherein ~~one of the~~ ~~at least one~~ chaotropic auxiliary agent is urea.

Claim 81 (currently amended): [[A]]The method according to claim 80, wherein the urea is present in a concentration between about 2 M and 8 M.

Claim 82 (currently amended): [[A]]The method according to claim 81, wherein the urea is present in a concentration between about 3 M to 6 M.

Claim 83 (currently amended): [[A]]The method according to claim 78, wherein the aqueous medium further comprises a mercaptan.

Claim 84 (currently amended): [[A]]The method according to claim 83, wherein the mercaptan is selected from the group consisting of dithiothreitol, dithioerythrol, 2-mercaptoethanol, cysteine, methyl thioglycolate, 3-mercaptop-1,2-propanediol and 3-mercaptopropionic acid.

Claim 85 (currently amended): [[A]]The method according to claim 83, wherein the mercaptan is 2-mercaptoethanol.

Claim 86 (currently amended): [[A]]The method according to claim 79, wherein the aqueous medium has a pH between about 8 and 10.5.

Claim 87 (currently amended): [[A]]The method according to claim 79, wherein the aqueous medium has a pH between about 9 and 10.

Claim 88 (currently amended): [[A]]The method according to claim 79, wherein the recombinant protein is present in the aqueous medium in a concentration between about 0.05 and 15 grams per liter.

Claim 89 (currently amended): [[A]]The method according to claim 79, wherein the recombinant protein is present in the aqueous medium in a concentration between about 0.5 and 5 grams per liter.

Claim 90 (currently amended): [[A]]The method according to claim 79, wherein the recombinant protein is present in the medium in a concentration between about 2 and 3 grams per liter.

Claim 91 (currently amended): [[A]]The method according to claim 78, wherein the recombinant protein is contacted with a mercaptan.

Claim 92 (currently amended): [[A]]The method according to claim 91, wherein the mercaptan yields less than 5 —SH radical of the mercaptan per cysteine residue of recombinant protein.

Claim 93 (currently amended): [[A]]The method according to claim 91, wherein sufficient mercaptan is provided to yield between about 0.07 to about 1.0 —SH radical of the mercaptan per cysteine residue of recombinant protein.

Claim 94 (currently amended): [[A]]The method according to claim 78, further comprising isolating a portion of the expressed recombinant protein which is in the bioactive conformation.

Claim 95 (currently amended): [[A]]The method according to claim 94, wherein the isolating is performed by ultrafiltration.

Claim 96 (currently amended): [[A]]The method according to claim 95, wherein the ultrafiltration is performed at a pH between about 8 and 11.

Claim 97 (currently amended): [[A]]The method according to claim 95, wherein the ultrafiltration is performed at a pH between about 9 and 10.

Claim 98 (canceled).

Claim 99 (currently amended): [[A]]The method according to claim 78, wherein the second human insulin precursor peptidyl fragment is capable of being bound by an anti-human-insulin antibody.

Claims 100-101 (canceled).

Claim 102 (currently amended): [[A]]The method according to claim 78, wherein

the second amino acid sequence of the human insulin precursor peptidyl fragment comprises
is the amino acid sequence of SEQ. ID. No. 4 SEQ ID NO:4.

Claim 103 (currently amended): [[A]]The method according to claim 78, wherein
the amino acid sequence of the second human insulin precursor peptidyl fragment comprises
is the amino acid sequence of SEQ. ID. No. 5 SEQ ID NO:5.

Claim 104 (currently amended): [[A]]The method according to claim 78, wherein
the second human insulin precursor peptidyl fragment comprises consists of the A chain and B
chain amino acid sequences of human insulin separated by and therebetween [[an]]a removable
amino acid sequence of between 1 and 34 residues in length.

Claim 105-112 (canceled).

Claim 113 (currently amended): [[A]]The method according to claim 78, wherein
the amino acid sequence of the first peptidyl fragment comprises is the amino acid sequence of
SEQ. ID. No. 1 SEQ ID NO:1.

Claim 114 (currently amended): [[A]]The method according to claim 78, wherein
the amino acid sequence of the first peptidyl fragment comprises is the amino acid sequence of
SEQ. ID. No. 2 SEQ ID NO:2.

Claim 115 (canceled).

Claim 116 (currently amended): [[A]]The method according to claim 78, wherein
the first peptidyl fragment is between 20 and 49 [[200]] residues in length.

Claims 117-119 (canceled).

Claim 120 (currently amended): [[A]]The method according to claim 78, wherein
the method further includes cleaving the at least one cleavable peptidyl fragment.

Claim 121 (currently amended): [[A]]The method according to claim 78, wherein
the at least one cleavable peptidyl fragment is an Arg or Lys residue.

Claim 122 (currently amended): [[A]]The method according to claim 78, wherein
the at least one cleavable peptidyl fragment is at least 2 amino acids in length where the and has

a C-terminal amino acid residue is selected from the group consisting of Arg and Lys.

Claim 123 (currently amended): A chimeric protein comprising from N-terminus to C-terminus:

a first peptidyl fragment, wherein the amino acid sequence of the first peptidyl fragment is ~~comprises~~ at least 60% identical to the amino acid sequence of at least the first 20 N-terminal amino acids of SEQ ID NO:1; and wherein the first peptidyl fragment is capable of being bound by an anti-human-growth hormone antibody;

a second human insulin precursor peptidyl fragment comprising consisting of a human insulin precursor which exhibits insulin-like bioactivity when folded in a bioactive conformation; and wherein the human insulin precursor peptidyl fragment comprises the A chain and the B chain of human insulin; and wherein the A chain and the B chain are separated by a removable peptidyl moiety of between 1 and 34 residues in length, and

an arginine or lysine residue or at least one cleavable peptidyl fragment linking the first peptidyl fragment and second the human insulin precursor peptidyl fragments fragment;

wherein the first peptidyl fragment is capable of mediating, upon contacting of the chimeric protein with a chaotropic agent, the formation of a correctly folded conformation of the human insulin precursor peptidyl fragment mediates folding of the second peptidyl fragment to cause the second peptidyl fragment to adopt the bioactive conformation.

Claim 124 (currently amended): [[A]]The chimeric protein according to claim 123, wherein the amino acid sequence of the second first peptidyl fragment is an amino acid sequence of SEQ ID NO:1 of the same length as the first peptidyl fragment. is capable of being bound by an anti-human insulin antibody

Claims 125-126 (canceled).

Claim 127 (currently amended): [[A]]The protein according to claim [[123]] 124, wherein the amino acid sequence of the second human insulin precursor peptidyl fragment comprises is the amino acid sequence of SEQ. ID. No. 4 SEQ ID NO:4.

Claim 128 (currently amended): [[A]]The protein according to claim [[123]] 124,

wherein the amino acid sequence of the second human insulin precursor peptidyl fragment comprises is the amino acid sequence of SEQ ID No. 5 SEQ ID NO:5.

Claims 129-130 (canceled).

Claim 131 (currently amended): A method of making a correctly folded human polypeptide with insulin bioactivity, said method comprising:

expressing a recombinant protein comprising, from N-terminus to C-terminus[[],]:
a first peptidyl fragment which has an amino acid sequence identical to the amino acid sequence of SEQ ID NO:1 through residues 1-20; which is at least 60% identical to a sequence of SEQ ID NO: 1 of the same length as the first fragment,

a second human insulin precursor peptidyl fragment which is comprising a human insulin precursor which exhibits insulin-like bioactivity when folded in a bioactive conformation; wherein the human insulin precursor peptidyl fragment comprises the A chain and the B chain amino acid sequences of human insulin separated by a removable peptidyl moiety between 1 and 34 residues in length, and

an arginine or lysine residue or at least one cleavable peptidyl fragment linking the first peptidyl fragment and second human insulin precursor peptidyl fragments, fragment;

wherein the first peptidyl fragment is capable of increasing the yield of the bioactive conformation of the insulin precursor formed upon contact of from contacting the protein with a chaotropic auxiliary agent as compared to the yield of the bioactive conformation of the insulin precursor formed from contacting the same protein lacking the first peptidyl fragment with the chaotropic agent;

contacting the recombinant protein with an aqueous medium comprising the chaotropic agent; and

cleaving at least one of the cleavable peptidyl fragments.

Claim 132 (new): The method of claim 78, wherein the recombinant protein consists of the first peptidyl fragment, the insulin precursor peptidyl fragment, and the linking peptidyl fragment, and wherein the first peptidyl fragment is identical in amino acid sequence to

an amino acid sequence of SEQ ID NO:1 of at least 20 amino acids in length or a sequence variant thereof having only a small number of amino acid substitutions.

Claim 133 (new): The method of claim 132, wherein the small number is zero or one.

Claim 134 (new): The method of claim 132, wherein the small number is two.

Claim 135 (new): The method of claim 132, wherein the small number is zero.

Claim 136 (new): The method of claim 78, wherein the human insulin A chain is identical in amino acid sequence to the amino acid sequence of residues 32-52 of SEQ ID NO:5 and the human B chain is identical in amino acid sequence to the amino acid sequence of residues 1-30 of SEQ ID NO:5.

Claim 137 (new): The method according to claim 133, wherein the human insulin precursor peptidyl fragment consists of an amino acid sequence identical to the amino acid sequence of SEQ ID NO:4 or SEQ ID NO:5.

Claim 138 (new): The method of claim 133, wherein the human insulin precursor peptidyl fragment consists of the amino acid sequences of the A chain and B chain amino acid sequences of human insulin and a removable amino acid sequence between 1 and 34 residues in length.

Claim 139 (new): The method according to claim 104, wherein the recombinant protein consists of the first peptidyl fragment, the insulin precursor peptidyl fragment, and the at least one cleavable peptidyl fragment.

Claim 140 (new): The chimeric protein of claim 123, wherein the chimeric protein is identical in amino acid sequence to the amino acid sequence of SEQ ID NO:6 or of SEQ ID NO:7.

Claim 141 (new): The chimeric protein of claim 124, wherein the human insulin A chain is identical in amino acid sequence to the amino acid sequence of SEQ ID NO:5 through residues 32-52 and the human B chain is identical in sequence to the amino acid sequence of SEQ ID NO:5 through residues 1-30.

Claim 142 (new): The chimeric protein of claim 124, wherein the protein consists of the first peptidyl fragment; the second peptidyl fragment; and an arginine or lysine residue or the at least one cleavable peptidyl fragment; and wherein the first peptidyl fragment is identical in amino acid sequence to an amino acid sequence of SEQ ID NO:1 of at least 20 amino acids in length and wherein the at least one cleavable peptidyl fragment is at least 2 amino acids in length and has a C-terminal amino acid residue selected from the group consisting of Arg and Lys.

Claim 143 (new): The chimeric protein of claim 124, wherein the first peptidyl fragment and the insulin precursor peptidyl fragment are linked by only one amino acid residue which is an arginine or lysine residue.